Remarks

The Office Action mailed August 19, 2009, has been received and reviewed. Claim 121 having been added, the pending claims are claims 75, 76, 78, 79, 82, 83 and 85-121. Reconsideration and withdrawal of the rejections are respectfully requested.

The 35 U.S.C. §112, First Paragraph, Rejection

The Examiner has rejected claims 75, 76, 78, 79, 82, 83, and 85-98 under 35 U.S.C. 112, first paragraph, alleging the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims. This rejection is respectfully traversed. In addition to remarks made in replies to earlier Office Actions, the Examiner is requested to consider the following.

The Examiner acknowledges that the specification is enabling for attenuating the expression of the disclosed target genes in the disclosed species. Applicants believe that the examples present evidence of the claimed invention using a sufficient number of genes and species of vertebrate cells in order to support the claimed genus. The standard is set out in M.P.E.P. § 2164.02, where it states that

> For a claimed genus, representative examples together with a statement applicable to the genus as a whole will ordinarily be sufficient if one skilled in the art (in view of level of skill, state of the art and the information in the specification) would expect the claimed genus could be used in that manner without undue experimentation. Proof of enablement will be required for other members of the claimed genus only where adequate reasons are advanced by the examiner to establish that a person skilled in the art could not use the genus as a whole without undue experimentation.

Applicants have demonstrated, by working, representative examples, the specific attenuation of gene expression using dsRNA in zebrafish embryos (Example I), chick neural crest tissue (Example II), and rat ROS cells[1] (Example III). In zebrafish embryos the targeted genes were GFP, T gene, Pax6.1, Nkx 2-7, and both T gene and Pax6.1 simultaneously. The HirA gene was

[1] As discussed in the Preliminary Amendment submitted April 28, 2003, rat ROS cells were used in Example III, not NIH 3T3 cells.

targeted in chick neural crest tissue, and a plasmid based GFP gene was targeted in murine (rat ROS) cells. It can be seen that the Applicants have shown successful gene silencing using dsRNA in a wide range of systems targeting a wide range of genes, and have provided numerous working examples. Vertebrate cells as diverse as fish cells, mammalian cells and avian cells were silenced. And these are merely the vertebrate organisms for which examples are provided; the specification further describes the target gene as being derived from any organism, with numerous examples of additional species being provided. It is submitted that the Applicants have made an adequate showing of enablement in the specification for the claimed genus.

The Examiner believes the results of the working examples are contradictory to what the skilled person would expect, and cites Oates et al. (Developmental Biology, 2000) and Zhao et al. (Developmental Biology, 2001). The Declaration Under 37 C.F.R. § 1.132 of Dr. Yin-Xiong Li, submitted herewith, states that Zhao et al. and Oates et al. do not show that injection of dsRNA causes degradation of endogenous non-target mRNA and nonspecific effects in zebrafish embryos. As stated in the Li Declaration, the phenotypes observed by Zhao et al. and Oates et al. can be explained as the result of physical damage to the embryo during injection. For example, both Zhao et al. and Oates et al. observed abnormalities that indicate there was physical damage to the embryo. Embryos depicted by Zhao et al. also showed leakage of cytoplasm and yolk, also indicating there was damage to the embryo. Thus, non-specific effects observed by Zhao et al. and Oates et al. could be the result of the physical damage to the embryos. Declaration under 37 C.F.R. § 1.132 of Yin-Xiong Li.

Moreover, Zhao et al. and Oates et al. use dsRNA formed from single-stranded RNA that has been purified by phenol/chloroform extraction (see, for example, page 216, first column of Zhao and page 21, first column of Oates). As stated in the Li Declaration, phenol is toxic to zebrafish embryos and causes concentration-dependent, adverse effects in zebrafish. Thus, non-specific effects observed by Zhao et al. and Oates et al. could be the result of toxic effects from residual phenol and/or chloroform in the mixture injected into the zebrafish embryos. Declaration under 37 C.F.R. § 1.132 of Yin-Xiong Li.

Further, Zhao et al. anneal sense and antisense RNA in 1.5 M sodium chloride and 0.667

M sodium bicarbonate. The chance of contaminating the embryo with sodium is greater, and it is well known that sodium in an injection solution is toxic for zebrafish embryonic development. Declaration under 37 C.F.R. § 1.132 of Yin-Xiong Li. Zhao et al. also expose the annealed RNA to conditions that may cause random degradation of the RNAs. These methods could result in the non-specific effects observed by Zhao et al. Declaration under 37 C.F.R. § 1.132 of Yin-Xiong Li.

In view of the Declaration of Dr. Li, the teachings of Zhao et al. or Oates et al. cannot be used as evidence of unpredictability.

The Examiner also states that it is critical to use suitable models to predictably extrapolate experimental results to other cell types, but the results observed in the working examples are not broadly applicable to all vertebrate cells (Office Action at page 6, last paragraph). In addition to using vertebrate cells as diverse as fish cells, mammalian cells, and avian cells, the Applicants have used an organism that is designed to be representative of the genus. The zebrafish is not merely a fish but is rather a model organism for studies of vertebrate organisms. In fact, experts have promoted the use of the zebrafish as a model for human biology. See "The Zebrafish Model Organism Database" available at http://zfin.org/zf_info/dbase/db.html.

Wianny et al. (Nature Cell Biology, 2000) is cited by the Examiner to further support the assertion that the results observed in the examples are not broadly applicable to all vertebrate cells. Wianny et al. state that "it is possible that the early mouse embryo is incapable of an interferon response and that there may still be difficulties in using RNAi at later stages" (p. 73, under Discussion). Despite the pessimism expressed by Wianny et al., Applicants have demonstrated that it is possible to achieve target specific regulation of gene expression in vertebrate cells by administering a double stranded RNA corresponding to the target gene. The skilled artisan could clearly apply that invention to other cell types of other vertebrate species given Applicants' data absent undue experimentation. In fact, Wianny et al. supports the surprising nature of Applicants' invention and is evidence that the claimed invention would not have been evident to the skilled artisan until they were presented with Applicants' data.

The Applicants note that there are large quantities of post-filing art that support predictability. The Examiner's attention is directed to, for example, the work of McCaffrey et al., who have used double stranded RNA to specifically inhibit luciferase expression in mice (McCaffrey et al., 2002, Nature, 418:39), Rossi et al., who have shown that dsRNA can be delivered in vivo by conjugation to cholesterol (Rossi, J., 2004, Nature, 432:155), the silencing of apolipoprotein B in mice by dsRNA (Soutschek et al., 2004, Nature 432:173), the lack of an interferon response to naked siRNA in animals (Heidel et al., 2004, Nature Biotechnology, 22(12), 1579), and the use of RNAi to inhibit neurodegeneration in polyglutamine disease in mice (Caplen N., 2004, Nature Medicine, 10(8):775).

The Examiner asserts that none of the working examples describe *ex vivo* treatment of a cell followed by implantation into any organism for any purpose (Office Action at page 4). The Examiner is requested to consider the disclosure of U.S. Provisional Patent Application 60/117,635 (the '635 provisional application). The '635 provisional application was incorporated by reference in the present application at the time of filing. The '635 provisional application exemplifies *ex vivo* treatment of chick neural crest tissue with dsRNA and re-implantation of the treated explant into embryos (see page 2 and Figures 7 and 8). Briefly, cardiac neural crest explants were treated with dsRNA specific for chick HIRA *in vitro*, implanted back into embryos after treatment, and the embryos were allowed to develop to embryonic day 8. Embryos in which the cardiac neural crest had been treated with the HIRA dsRNA manifested a significant increase in persistent truncus arteriosus (a heart defect) at day 8. Thus, the specification clearly enables an *ex vivo* method that includes treatment of an explanted cell with dsRNA followed by implantation into an organism. Moreover, this working example of successful *ex vivo* treatment followed by implantation is evidence that, in view of the teachings of the present specification, undue experimentation is not required to practice an *ex vivo* method to treat a disease or pathogen.

The applicants note that the presumption is that an application is enabled, and that this is overcome only if the Examiner can show that undue experimentation is necessary to use the invention as claimed. Furthermore, the mere fact that experimentation may be involved, and even

be complex, does not necessarily make the experimentation undue. The applicants do not believe undue experimentation would be necessary to practice the invention as claimed. It is well-established that some experimentation is often to be expected in unpredictable technologies, such as molecular biology. The question is whether the amount of experimentation needed to practice the invention, as claimed, is undue. This question is answered more readily if the method of the invention is broken down into separate steps. For the first step, a vertebrate cell is explanted from a vertebrate organism. Methods for accomplishing this are routine and known to those skilled in the art. Next, to attenuate the expression of a target gene in a vertebrate cell, the nucleotide sequence of the gene can be obtained either from a database or from routine procedures used to determine the sequence of a gene. RNA capable of hybridizing to the target gene is then synthesized, again using routine methods known to those skilled in the art, and the dsRNA is supplied to a vertebrate cell using methods which are again routine and known to those skilled in the art. Finally, the cell is implanted into a vertebrate organism, using routine methods known to those skilled in the art. It is thus respectfully submitted that the pending claims are enabled. Reconsideration and withdrawal of the rejection of the pending claims under 35 U.S.C. §112, first paragraph, is accordingly requested.

The 35 U.S.C. §103 Rejection

The Examiner rejected claims 75, 76, 78, 79, 82, 83, and 85-120 under 35 U.S.C. 103(a) as being unpatentable over Fire et al., in view of Ekenberg et al. This rejection is respectfully traversed. In addition to remarks made in replies to earlier Office Actions, the Examiner is requested to consider the following.

A rejection under 35 U.S.C. §103 is based on a document that is considered prior art under 35 U.S.C. §102. M.P.E.P §2141.01(I). A reference must contain an enabling disclosure to qualify as prior art under 35 U.S.C. §102. M.P.E.P. §2121.01. On the other hand, a reference disclosing an inoperative device is prior art for all that it teaches, and a non-enabling reference may qualify as prior art for the purpose of determining obviousness under 35 U.S.C. §103. M.P.E.P §2121.01(II).

The applicants respectfully assert that Fire et al. does not provide an enabling disclosure for vertebrate cells. Mere passing reference to embryonic stem cells (col. 9, lines 44-48), zebrafish (col. 8, lines 35-37) and vertebrates (col. 8, lines 35-37) does not enable the attenuation of gene expression in embryonic zebrafish cells or vertebrate cells, any more than Fire et al.'s listing of over 100 types of cancer on column 10, line 28 to column 11, line 4 provides an enabling disclosure for cancer treatment. The only detailed description of work done by Fire et al. was in *C. elegans.* This does not provide an enabling disclosure for embryonic zebrafish cells or vertebrate cells. Not only is *C. elegans* an invertebrate, but it is a primitive and simple invertebrate. It is only 1 mm long, includes only 959 somatic cells, and is often handled as a microorganism; for example, it is usually grown on petri plates seeded with bacteria. The description of dsRNA administration to this single, simple, invertebrate organism does not provide an enabling disclosure for the claimed methods in embryonic zebrafish cells or vertebrate cells.

Since Fire et al. does not provide an enabling disclosure, it is not available for use as prior art under 35 U.S.C. §103 for determining obviousness. Since Fire et al. is not available for as prior art under 35 U.S.C. §103 for determining obviousness, a *prima facie* case of obviousness has not been made.

Further, the Examiner is requested to note that at the earliest priority date of the present application, the state of the art suggested that dsRNA would not work for silencing gene expression in vertebrate cells. In particular, the Examiner is requested to note that Fire, a leading researcher in the field, expressed doubt about the applicability of this technology to vertebrates. In a review published September, 1999, 8 months after the earliest priority date of the present application, Fire stated:

> "[f]rom a technical perspective, one could certainly hope that RNA-triggered silencing would exist in vertebrates . . . . Although this hope is not ruled out by any current data, *the simple protocols used for invertebrate and plant systems are unlikely to be effective*."

(Fire, Trends in Genetics, 15:358-363, 363 (1999); emphasis added). Nothing could be more clear than the statements made by Fire. Yes, there was hope, but even in that hope there is doubt of the existence of RNAi in vertebrates, and further doubt that it would allow medical applications, and even further doubt that the tools available would be effective. Even nine years later, while recounting his work in RNAi, Dr. Fire noted in his Nobel lecture that:

> Despite the great enthusiasm from those of working with plant, worm, and insect model systems, the mechanism by which dsRNA could silence gene expression was still an unknown. Seminar slides made at the time would show dsRNA and the mRNA target somehow entering a large and mysterious "black box", followed by degradation of the target RNA and some unknown fate of the effect dsRNA. This "black box" explanation limited our grasp of the RNAi system, both for understanding the underlying biology and for applying RNAi to organisms (like humans) where the response to dsRNA was more intricate than for "simple" invertebrates.

(Andrew Fire Nobel Lecture, GENE SILENCING BY DOUBLE STRANDED RNA, December 8, 2006, 198, 212, *Les Prix Nobel. The Nobel Prizes 2006*, Editor Karl Grandin, [Nobel Foundation], Stockholm, 2007.

Dr. Fire makes it clear that despite work in the genetics in plants, fungi, and flies, at the time of his work, he and his colleagues were not in a position to even design experiments to explore vertebrate systems. Specifically, he states, "[d]espite these hopeful suggestions, the analysis had not put us in a position either to propose a unifying mechanism for RNAi or to design experiments to test for the efficacy of the systems in mammals." (*Id.,* at 215). Thus, there was an art-accepted skepticism as to the workability of RNAi in vertebrates both at the time of the Fire references and in recollections of Fire himself.

The comments made by Dr. Fire clearly indicate that the state of the art *teaches away* from the claimed invention.

The present inventors were the first, in this highly competitive art area, to demonstrate success in attenuating gene expression in target vertebrate cells using dsRNA. Indeed, the state of the art suggested that dsRNA could not be successfully used for specific attenuation of gene expression in vertebrates. Gene silencing technology based on dsRNA, as it relates to vertebrates, was not in the hands of the public until the present inventors developed a method

that caused attenuation of gene expression in a vertebrate system.

For at least these reasons, reconsideration and withdrawal of the present rejection is respectfully requested.

Summary

It is respectfully submitted that the pending claims are in condition for allowance and notification to that effect is respectfully requested. The Examiner is invited to contact Applicants' Representatives at the telephone number listed below if it is believed that prosecution of this application may be assisted thereby.

Respectfully submitted
By
Mueting, Raasch & Gebhardt, P.A.
P.O. Box 581336
Minneapolis, MN 55458-1336
Phone: (612) 305-1220
Facsimile: (612) 305-1228
Customer Number 26813

Date July 19, 2010

By: David L Provence
David L. Provence
Reg. No. 43,022
Direct Dial (612) 305-1005

CERTIFICATE UNDER 37 CFR §1.6:
The undersigned hereby certifies that this paper is being transmitted via the U.S. Patent and Trademark Office electronic filing system in accordance with 37 CFR §1.6(a)(4) to the Patent and Trademark Office addressed to the Commissioner for Patents, Mail Stop RCE, P.O. Box 1450, Alexandria, VA 22313-1450, on this 19 day of July, 2010.

By: Sandy Truehart
Name: Sandy Truehart